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One of the troubling physiological problems for the use of concentrated hemoglobin solutions in resusitation has been fear of the consequences of extracellular hyperonconicity. Here, we will review the theoretical and some of the clinical aspects of this problem and argue that if compartmental membranes are not compromized the effects will be transient and without long term consequences.

Oncotic pressure results when two fluid compartments are separated by a membrane that is impermeable to one or more solutes in either or both comparments. The potential is for solvent to move to equalize the concentration of the impermeable solutes. This potential is exactly equivalent to hydrostatic pressure, and thus we have the Starling Hypothesis that suggests that systolic pressure in the capillary (18 to 28 mmHg) is balanced by a higher concentration of protein in the plasma than in the interstitium.

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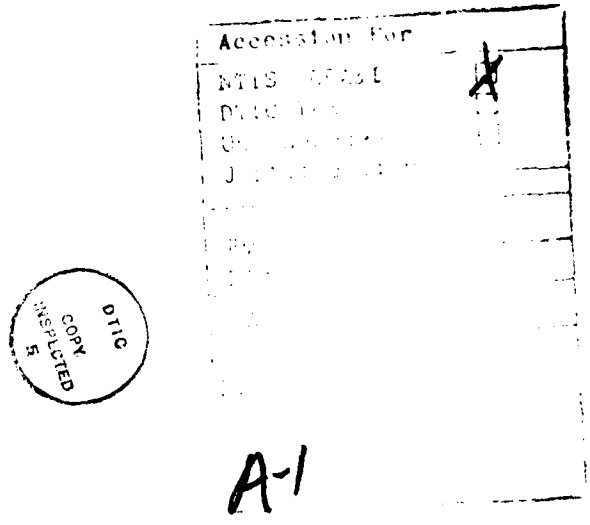
19. Abstract (continued)

Frequently this balance is upset, either pathologically or iatrogenically. Therapies consisting of NS-D5 or NS-D10, being respectively two and three times normal osmolarity, temporarily expand the vascular volume until the glucose, with a half-time of 15-20 minutes, can diffuse into the interstitium and be taken up by the cells. Hypertonic saline solutions, now under study, act similarly but with a shorter half-time, 5-10 minutes. Glucose tolerance tests will raise blood sugar to 200 mg-%, about 10 meq/l (and diabetics may show with sugar levels of 500 mg-%). While this test sugar is excluded from the cells, it will exercise oncotic pressures of 5 times that of normal plasma protein. Now these situations may not be innocuous (particularly if chronic), although under normal circumstances we believe them to be. The Hb solutions are not out of the range of usual procedures. Hyperoncotic Hb-tetramer solutions of 14 mg-% are only about 2 mosm, on the orde of twice that of plasma protein and less than half the concentration of Hb in erythrocytes.

Although absolute oncotic pressures can be quite high-- about 2*C +.2*C**2+.01C**3, where C is protein in gm-% --the cells are "protected" by the fact that through the agency of the cation pump, Na is excluded from the cell, i.e., acts like an oncotic paritcle. The sum of all of the "oncotic" solutes both inside and outside of the cell is thus very high and the relative change in onconicity by the change of a few mosm outside will have a proportionately smaller effect on the cell. The Gibbs-Donnan forces (electrical neutrality) also play a role (e.g., forcing K into the cell), and it is believed that the cell also responds by allocating an increased amount of energy to the active pump (with the tendency to maintain cell volume).

If the compartmental membranes are patent, not modified by toxic, inflammatory or pyrogenic reactions, it is likely that the physiology is not harmed by temporary, expansions of vascular or interstitial spaces on the order of those seen in Hb solutions. The physical forces described here can be simulated on a computer and the volume changes predicted. We can not yet predict the inotropic stress on the heart, nor with any assurance, the volume changes in the brain. To make better predictions of other organ responses, it is critical to know how the tetramer is catabolized.



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Edward O. McCord 21 June 89

PI Signature Date

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Introduction.

Our laboratories have, for some time, been interested in the potential of a modified hemoglobin molecule in solution to serve as a reperfusion solution in hemorrhage, and, generally, as a versatile blood substitute in similar situations, for example, surgical loss. A variety of resuscitation fluids, from crystalloids to fluorocarbons, have been recommended for treatment of acute hemorrhage in spite of the clinical dictum: "Give them what they lost.", namely, whole blood or plasma. This basic principle, undoubtedly a good one, is nevertheless often superseded by the exigencies of circumstance or dictated by an aspect of the presentation, and the search for an ideal blood substitute continues.

One of the troubling physiological problems for the use of concentrated hemoglobin solutions in resuscitation has been fear of the consequences of extracellular hyperonconicity. A problem may arise as a consequence of upsetting the delicate balance, particularly across the capillary wall, that has come to be known as the Starling Hypothesis: the vascular mean systolic pressure, tending to cause the capillaries to leak, is just balanced by the oncotic pressure of the plasma, which is higher than that of the interstitial fluid. However, the hemoglobin, with a molecular weight of 64,000 dalton (approaching that of serum albumin's 68,000), is an oncotic species and therefore a plasma expander. Normal plasma proteins have a concentration of less than one meq. per liter of plasma, whereas 14 gm/dl of hemoglobin would have a concentration of more than 2 meq. per liter and the question arises whether this additional plasma oncotic pressure could have deleterious physiological effects. A vascular hypervolemia at the expense of interstitial and intracellular volumes caused by increasing plasma oncotic pressure is to be avoided, as is an interstitial edema, caused by reducing the plasma oncotic pressure or increasing intravascular pressure.

To put this problem in perspective, several other common clinical therapies also modify plasma oncotic pressure, sometimes drastically, and also, a variety of clinical problems result in acute or chronic maldistribution of fluid volume. A common reperfusion fluid of choice is crystalloid such as Ringer's (Shires, 1973) which necessarily dilutes the plasma oncotic pressure or, conversely, concentrated serum albumin (Randall, 1975) or concentrated saline (Hannon and Wade, 198x) may be administered to raise plasma volume. Also, high blood pressure, congestive heart failure, diabetes, and a variety of other syndromes are characterized by, among other things, maldistributed volume accumulation.

Here, we review the theoretical and some of the clinical aspects of this problem using a standard swine hemorrhage model with physiologic fluid replacement. We argue that a properly constituted hemoglobin solution purified of toxic, pyrogenic, and inflammatory factors is no more harmful than other common clinical reperfusion procedures and is beneficial in that it also carries oxygen. If compartmental membranes are not compromised the effects will be transient and without long term consequences.

Methods

a. Animal Model.

The Division of Military Trauma Research has developed and characterized an immature swine model (Hess, 198x) of hemorrhagic shock that mimics human physiology in significant ways such as cardiovascular response and blood and body composition. The swine are large enough to allow invasive instrumentation, extensive blood sampling, breath by breath analysis by snout mask, and cardiac output measurement. In the course of model development and preliminary fluid testing, a control physiologic data base has been obtained in the basal state, when exercised on treadmills, restrained in pavlov slings, and when splenectomized. Splenectomy is necessary because the pig, like the dog but unlike man, can mobilize erythrocytes sequestered in the spleen (Hannon, 1985).

Eight immature (20-25 kg) female Duroc/Yorkshire cross swine were used in the experiment. Each animal was sedated (0.08mg/kg atropine, 2.2 mg/kg ketamine, and 2.2 mg/kg xylazine), and then splenectomized using aseptic techniques on the morning of the experiment under general endotracheal anesthesia (Halothane, nitrous oxide, and oxygen). Aortic and venous catheters were placed using established procedures.

Anesthesia was maintained throughout the full period of surgical preparation, exchange transfusion, and observation of oxygen transport function. The animals were then recovered and followed with physiologic measurements and blood studies using small samples drawn from the implanted catherters. Daily antibiotics were given via the venous catheter. Eight animals were used in the expectation that two would be euthanized (as described below) at two days, two more at four days, two more at ten days, and perhaps two would be lost as technical failures.

The modified hemoglobin solution (bis (3,5-dibromosalicyl) fumarate modified hemoglobin, or DBBF) is provided by Baxter Healthcare Corporation at 14 gm/dl in Ringers lactate adjusted to pH 7.4 with HCl of NaOH as required. The hemoglobin, extracted from outdated blood, is purified and the molecules are cross-linked by standard procedures to prevent dimerization. Erythrocytopheresis and DBBF solution infusion was performed as an exchange transfusion using the Fenwal PS400 plasma separator with the tubing modified to collect cells and return the filtered plasma mixed with the DBBF hemoglobin solution in volume equal to the removed blood. The one compartment model of pheresis of a 21 kg pig with pheresis at 70 ml/min of blood and returning all the plasma as filtrate predicts that processing of two and one-half complete blood volumes would result in a pig with a packed cell volume of 0.03 and a plasma hemoglobin of 11 gm/dl with infusion of only 2.0 liters of hemoglobin solution. About 70 ml/Kg of original blood volume (based on red cell content) is removed.

Three milliliter blood samples were drawn every two minutes during the exchange phase, every five minutes for twenty minutes post exchange, followed by four half-hourly samples, six hourly samples, then daily until euthanasia. After three hours, the animals were recovered from anesthesia and observed for two to ten days. Electrocardiographic and hemodynamic monitoring by the implanted catheters was maintained throughout. Blood analysis included cell counts, plasma and total hemoglobin and percent met, electrolytes, blood gases and pH, osmolarity and onconicity, lactate and lactic dehydrogenase, creatinine and creatine kinase, and clotting studies. Hepatic function and breakdown tests (albumin, bilirubin, SGOT and LDH), and kidney funtion tests (BUN, creatinine, volume) were performed at least every day.

Upon completion of the experiments, from 2 to 10 days after exchange, the pigs were anesthetized by intravenous barbituate via the implanted catheters. The carotid artery was catheterized and the brain perfused with 10% Formalin. The eyes were removed and fixed in Karnovsky's solution for light and electron microscopy. A small piece of liver, lymph node, and renal cortex were minced in similar fixative. The lungs were removed and the right diaphragmatic lobe fixed by transbronchial perfusion of

Karnovsky's. A routine gross necroposy was performed. Tissue samples of visceral organs were fixed and examined by light microscopy for the presence of iron pigments or fibrin tangles.

b. Computer Simulation.

The computer simulation program is designed to compute the product composition of any chemical reaction system under a variety of constraints. Technically, the program calculates the (Gibb's) minimum free energy function following the theoretical development of White (1958). The program structure was developed in Clasen (1963, 1965), and subsequently evolved into a physiological research tool used in a variety of applications (DeLand, 1966, 1971, 1972). In physiology, it is usually assumed (but not necessary) that intracompartmental chemical reactions are much faster than intercompartmental transport so that each compartment is near equilibrium and is driven in time by membrane transport and constraints. A model of the physiological system under study will contain all of the chemical reactions and compartments deemed essential to expose the detailed mechanisms, and will then be driven under the protocol of the corresponding wet laboratory experiment.

Briefly described, this model contains the four fluid compartments, plasma, redcells, interstitium, and intracellular (muscle and two simple subcompartments for heart and brain volume calculation). In each compartment we calculate protein buffering of H+ ion, the bicarbonate system, phosphate ionization, electrolyte composition, osmolarity, onconicity, and a variety of derived data such as serum albumin chloride and calcium binding, hemoglobin binding of the ligands O2, CO2, DPG, Cl-, the Starling effect, and protein charge. The cellular compartments, are assumed to be perfect osmometers; water will move freely (and quickly) among all compartments to equilibrate osmolarity while the oncotic solutes and mean systolic pressure exert the appropriate potentials. The Gibbs-Donnan equations ensure that each compartment is electrically neutral, and the active cation pumps are simulated by an equivalent work function transporting cellular cations up to specified gradients.

Under any (physiologic) conditions of gas composition and total fluid and electrolyte content, a calculation will predict the (steady-state) distribution of volumes and electrolytes in each compartment under the simultaneous constraints mentioned above including the chemical reactions. Beginning with the "control" composition, distribution, and body weight, an experiment may be performed following the identical protocol of a corresponding animal experiment. Gas composition changes, fluid

additions or deletions, temperature changes, and other normal procedures may be simulated, and after initial validation of the simulation, future results may be predicted.

c. Protocol.

Reperfusion with hyperoncotic hemoglobin solution will result in vascular expansion greater than the volume of fluid added, the excess coming from the surrounding interstitium and intracellular fluid compartments. To simulate this behavior, the wet laboratory protocol will be followed incrementally, that is, the mathematical model of the 22 Kg pig will be exchanged in 70 ml increments until the plasma HbX content is approximately 8 gm/dl, which should require about 45 simulated minutes, as in the wet laboratory. We note that the second and subsequent increments removed will contain an increasing fraction of DBBF and a decreasing fraction of erythrocytes. Because of onconicity, it will also contain a varying fraction of interstitial fluid, depending upon the composition of the perfusion fluid. Part (about half) of the separated plasma fraction is reintroduced along with the DBBF, and also, intracellular fluid should begin to diffuse into the interstitium as evidenced by increasing interstitial potassium.

Using the observed intake/output after the exchange period, the volume of intake or loss are periodically added to the model. Also, using the observed plasma DBBF decay curve, DBBF is moved into the interstitium and then deleted from the model following an hypothetical single exponential catabolism curve with a half time of about 11 hours. With each incremental change, a new volume distributions and electrolyte compositions can be calculated, but there is very little experimental data against which the metabolism of DBBF can be calibrated.

We will compare these calculated results with that observed in the mean of eight animals and also with the results of similar fluid protocols taken from the clinical literature. In particular, the DBBF results will be compared to administration of Ringer's lactate alone, 25% serum albumin, 6% mmw Dextran, hypertonic (7.5%) saline, and hyperglycemia under the experimental conditions and in hypernatremia and hemorrhage..

Results

Detailed results of the eight animal experiments are reported in a companion paper (Hess and Winslow, 198x). Here, we require enough detail of the animal results to validate the mathematical simulation. The volumes of plasma and count of red cells, plasma electrolytes, hematocrit, plasma DBBF, and total Hb were among the data observed, and will be used. First we will test the simulation and then use it to extrapolate to related experiments.

a. Control values.

The simulation calculations begin at the observed control values. Standard body composition data for the swine are taken from the exhaustive studies of Bossone and Hannon (Bossone, 1985), which determine the volumes and significant electrolyte compositions of fluid compartments for the Duroc/Yorkshire breed. The mean weight of eight animals was 22 Kg. The 22 Kg animal has a plasma volume of 1.1 liters (Hct = 27%), an interstitial space of 2.17 liters, and intracellular space of 8.33 liters, Table 1. Because of the relatively low hematocrit, blood hemoglobin is only 8.7 gm/dl, less than two-thirds that of h man, although the hemoglobin saturation curve and p50 is similar (Hess and Winslow, 198x). The electrolytes were normal, which in the pig means values similar to human (for both plasma and erythrocytes). The pH of the perfusion fluid is adjusted to 7.4 with HCl or NaOH as required. The pH of both arterial and venous plasma drift slightly alkalotic during the animal exchange transfusion, for example, the arterial pH changes from 7.38 to 7.395. But, since the standard error of the mean of the pH measurement was 0.015, in the simulation the pH is held constant. The electrolytes are not significantly altered, although lactate ion increases, probably owing to the input of lactate ion in the DBBF solution.

The simulation, in this case, took 25 steps of 70 ml each in which the extracted cells were discarded and 60% of the plasma was returned. At each iteration the new distribution of body fluids was calculated so that successive extractions contained the gradually changing mix of vascular fluid, including fluids from the interstitium and intracellular space that may have been transported under the changing conditions. About 70 percent of the red cell hemoglobin, and by inference red cells, were removed, and about 70 percent of plasma remained (with recirculation). Total hemoglobin (plasma DBBF plus red cell) remained the same in the laboratory, but rose from 8.7 to 8.9 gm/dl in the simulation. Hematocrit in the simulation was 10.1 % at the end of reperfusion, but 7.9 in the laboratory. This discrepancy arises because the laboratory

hematocrit is calculated from the (Colter) cell count whereas the simulation measures the slight change in cell volume.

The observed time-related changes of plasma volume, red cell count, and total plasma hemoglobin (including DBBF) for the mean of eight animals is illustrated in Fig. 1 and listed in Table 1. The simulated changes are also shown, well within the variance of the eight animals. This simulation appears to emulate the time dependent reperfusion model within measurement error. We now turn attention to a more detailed analysis of the system at the end of transfusion. The groups of bars in Fig. 2 and 2a display the volumes of the principal fluid compartments: plasma, erythrocytes, interstitial and intracellular space, under several conditions. (Figures 2 and 2a display the same experiments, but on different scales.) Also derived are the hematocrit, total blood volume (including protein volume), the plasma DBBF, and blood total hemoglobin, Table 2.

The first two groups in Fig. 2 indicate the control volumes calculated and compared with data from Bossone and Hannon. The third group simulates the volumes before interstitial reflow of the hemorrhaged animal, and the fourth group of Fig. 2 displays the simulated volumes at the end of a transfusion with DBBF following the above protocol, where only the plasma volume was actually observed (calculated by the method above). The total blood volume (plasma plus red cells) has increased over control from about 1.52 liters to about 2.26 liters, about 660 ml or 43 percent, of which approximately 140 ml is from the cells (including red cells) and the remainder is from the 1.4 liters of fluid added (including recycled plasma). This is an excess of about 860 ml over the normal total control volume.

If a fraction of the plasma had not been recycled, the vascular volume would have been considerably smaller, almost 600 ml, fifth group of Fig. 2. The Hb concentration would be 36 percent greater, Table 2, but hemoglobin content would, of course, be the same. The final group of bars in Fig. 2 is a comparison of the oncotic effect of DBBF reperfusion with hyperglycemia, a common clinical problem. This patient had a blood glucose of 500 gm/dl, or about 25 meq glucose/liter (created by adding glucose in 250 ml water). In the simulation, we held the glucose concentration ratio in the extracellular to intracellular fluids at 10:1, which raised the plasma volume to 1.893 liters, about the same as the DBBF reperfusion. The actual volumes are quite sensitive to this ratio, however, and the cells are likely to have exhausted this substrate, making the ratio and the extracellular volumes greater.

b. Alternate reperfusions

We next wished to test the sensitivity of this system to a variety of parameters. The electrical charge of the infused oncotic DBBF can be critical (Veech, 1986); at pH 7.4 DBBF, according to its titration curve (Marini, 198x), would have an average charge of about -4 per molecule, which is the charge used in the simulation. By forcing the DBBF to have zero charge, the second group of Fig. 3, the Gibbs-Donnan forces do move fluids somewhat, but not appreciably, Table 3. Later, we observe serum albumin, with a charge of about -15, and the effect is considerably larger. The trend is for increased extracellular volume at the expense of the intracellular as the protein becomes more negative.

In the simulation, Ringer's chloride is the solvent for DBBF, however, the lac- ion in Ringer's lactate can be represented as HCO3- ion, the product of metabolism of lactate, and it is assumed that the pulmonary system can carry off excess CO2. Therefore, substitution of HCO3- for Cl-; will have an alkalinizing effect. In the next group of bars in Fig. 3, the pH rose from 7.38 to 7.43 when lac- was used in the DBBF solution instead of Cl-. The effect of higher pH is to decrease plasma volume, however this is not seen in the pig because acid/base equilibrium is not achieved until well after the perfusion period. Normal saline (and Ringer's chloride) is actually about 40 percent high in the potentially harmful Cl- ion (Veech, 1986). On the other hand, Ringer's lactate supplies an alkalinizing anion that is already likely to be overly abundant in hemorrhaged patients. Ringer's acetate is, perhaps, another possibility.

Clinically, a 70 percent red cell loss, as in the DBBF exchange transfusion, would not be tolerated without immediate replacement. In the DBBF exchange, the loss is about 50 ml/Kg or about 1.1 liters, without recycling plasma and before interstitial reflow. Next, in Fig. 3, fluid redistribution under the DBBF protocol is compared to other plasma expanders and reperfusion fluids. First, 1 liter of Ringer's is added alone to the hemorrhaged pig (instead of the DBBF solution), then 250 ml of 25% serum albumin solution (sodium albuminate) is added in addition to the Ringer's, and, finally, 250 ml of 1-molar saline (in 6% dextran) is substituted for the serum albumin. All are given as a bolus to the model.

These solutions increase plasma volume to 1.13, 1.50, and 1.49 liters respectively (Table 3) which is from about normal for the animal to 5 percent greater than the DBBF increase. Ringer's alone, however, expands both extracellular compartments (compared with the 70% blood loss column of Table 2), because it is approximately isotonic with them, without appreciably disturbing the intracellular spaces. The red cell compartment is increased as a consequence of the excess chloride from Ringer's chloride. The hypertonic saline in dextran (average molecular weight

70,000) significantly expands the extracellular space at the expense of the intracellular because of the osmotic shock and because the dextran is oncotic in plasma. However, whereas the Ringer's alone expanded both extracellular compartments proportionately, the oncotic dextran causes a disproportionate increase in plasma; though it is not a great effect since 6% dextran is only about 1 mosm per liter and only 1/4 liter was added. The serum albumin (molecular weight 68,000) almost doubles the plasma compartment, to 1.5 liters, and, with the liter of Ringer's, also increases the interstitium. It increases the intracellular space slightly also (because of its negative charge and the excess chloride from Ringer's). All of these fluids increase vascular volume, and probably systolic pressure, but they result in only 2 to 3 gm/dl of hemoglobin (Table 3) compared to the DBBF perfusion of 9.2 (Table 2).

c. Hypertonicity and hemorrhage.

Figures 4 and 5 (and corresponding Tables 4 and 5) refer to comparisons of the DBBF reperfusion with other reperfusion choices for clinical hypertonicity and hemorrhage. The simulated hypertonicity resulted from a loss by the 22 Kg pig of 1.5 liters of the clinical 1/4 normal saline (that is, 35 meq NaCl/l); the plasma sodium is 175 meq/l and is treated first with 1.5 liters of Ringer's chloride. The hemorrhage resulted from a loss of 37.5 ml blood per Kg total body weight, or about 825 ml blood, and is also treated first with 1 liter Ringer's. Subsequently, both are treated with either 250 ml of 25% serum albumin (sodium albuminate), 250 ml of 1-molar NaCl (in 6% dextran), or 1.25 liter of the 14 gm/dl DBBF solution (without the prior Ringer's).

In both hypotonic loss and hemorrhage a liter of Ringer's is added, in the first case to safely bring down the high plasma sodium and in the second case just to increase volume. Ringer's solution alone expands the extracellular spaces, distributing between the plasma and interstitium approximately in proportion to their original volumes, but because the crystalloid dilutes the remaining protein, reducing extracellular oncotic pressure, a small volume is taken up by the cells (Tables 4 and 5). An osmotic expansion of the red cells of about 20 percent in the hypertonic animal and 30 percent in hemorrhage is a consequence, in the both cases, of the chloride ion in excess of the normal plasma concentration, and exacerbated, in the case of hemorrhage, by the small capacity of the red cell compartment.

The effects, in Figs. 4 and 5, of serum albumin and DBBF are somewhat comparable, primarily increasing the plasma volumes significantly and increasing intracellular spaces only slightly compared to Ringer's alone. This experiment illustrates, however, the contrasting effects molecular charge and numerical

onconicity. The 25% serum albumin is about 4 mosm/l so that in 250 ml, about 1 mosm of plasma onconicity was added which is slightly more than the normal plasma content of about 0.8 mosm/l. In the liter of DBBF (without prior Ringer's but in Ringer's solution), slightly more that 2 mosm onconicity are added, but its charge, at pH 7.4, is only -4 per molecule compared to -15 for albumin. In consequence the DBBF tends to accumulate the volume in the plasma, while albumin increases the volume in all compartments, including the intracellular. The albumin, as it is usually administered, is significantly less effective as a plasma expander than would be DBBF because it is less concentrated and has a relatively high electrical charge, but on the other hand, only 250 ml of DBBF on top of a liter of Ringer's would have only a fraction of the oncotic effect seen here.

The 1-molar saline is made up in a 1 mosm medium-molecular-weight dextran, and is therefore very efficient at expanding the extracellular space at the expense of intracellular. A small fraction of this expansion is due to the oncotic dextran as may be seen, for example, in Table 6 where the effect of dextran alone is also shown. The NaCl, like Ringer's, distributes into the interstitium and, without dextran, would be distributed proportionate to the original plasma/interstitial volume ratio. The very high NaCl concentration (185 meq/l in the hypertonic patient and 165 meq/l in the hemorrhage) decreases the intracellular space remarkably; the volume decreases 0.6 and 0.8 liters for the hypertonic and hemorrhage, respectively, compared to Ringer's alone. Of course, hypertonic saline would not be recommended for the hypertonic patient; but can be used here to illustrate the principles of its action.

In hemorrhage, the therapeutic fluids are again supplemented by additional crystalloid since the fluid resources of the body have been depleted. Accordingly, the total fluid given after a 37.5 ml/Kg blood loss was 1.25 liters (blood volume of the 22 Kg pig is about 1.1 liters). Again, the 250 ml of 25% albumin contributes only slightly more than a normal oncotic protein concentration, and plasma volume therefore increased about 0.82 liters, that is, to about normal. DBBF draws excessive volume into the plasma, but concentrated saline, again filling the extracellular volumes proportionately, fails to fill the plasma space. In all cases, of course, the DBBF raises the hemoglobin content to above normal for the pig.

d. Toploading.

In Fig. 6 (and Table 6), the results were obtained by top-loading the 22 Kg pig with the same fluids as in the previous experiment (but omitting the liter of Ringer's), and 1

mosm mwm dextran alone was added in 250 ml of water to display the effects of the neutral oncotic particle.

The first simulation experiment was to top-load the animal with 250 ml of the concentrated sodium albuminate in water solution, not an uncommon procedure in cardiogenic pulmonary edema except that the solvent is normally 0.9 gm/dl NaCl. The plasma compartment increased about 340 ml, about 30%, at the expense of interstitial volume; hematocrit (Table 6) goes down by about 25 percent. Had the solvent been normal saline instead of water the increase in plasma volume would be 540 ml (not shown) because the additional Na+ is an external solute. This amount of albumin is almost isooncotic, that is, one-fourth liter contains only about 1 mosm albumin, so the volume of plasma increased only slightly over the volume of the solution added. Also, an anomalous change in the intracellular volume occurs (it increases by about 130 ml) owing to osmotic dilution of the extracellular space.

Hypertonic saline solutions have a quite different response. A top-load of 250 ml of 1-molar saline in 1 mosm dextran increases the plasma volume by about 70 percent to 1.53 liters from the control 1.11 liters. while the intracellular volume decreases by about 0.7 liters, The greater share of the added NaCl solution is retained in the plasma, compared with control or with Ringer's alone, because of the oncotic dextran. Concentrated saline without dextran distributes in the extracellular volume uniformly, while 1 mosm of dextran in one-fourth liter of water causes a disproportionate share to accumulate in plasma. To see this separate effect, the 6% dextran solution was top-loaded on the animal (Fig. 6 and Table 6). The plasma volume increased by about 150 ml primarily at the expense of the interstitium. The effect of the sum of the separate saline and dextran administrations is not identical to the combined hypertonic saline and dextran since, for example, the effect of dextran on the normal state will not be the same as the effect on the hypertonic state. In every case the result depends upon the initail conditions.

Finally, one liter of the concentrated, 14 gm/dl (2.18 meq/l) DBBF solution was added to the normal animal. An entire liter was used because it follows the laboratory protocol for toploading, and just to examine the results of this extreme case. This is over twice the onconicity of the normal plasma, but the DBBF average charge of -4 much smaller than the normal plasma proteins and is neutralized with H+ ion in contrast to the Na+ used for the -12 charge of sodium albuminate. The plasma volume increases only about 800 ml over control (Fig. 6), less than three times the effect of 250 ml of serum albumin, but like albumin, the intracellular space does not change volume appreciably.

This result would be similar to administration of concentrated serum albumin but for the fact that the hemoglobin molecule also carries oxygen. The result is quite different from that obtained by administration of crystalloid (which expands both plasma and interstitium) or concentrated normal saline (which also expands extracellular volume but at the expense of intracellular space). The hemoglobin solution thus tends to dry the interstitium and slightly concentrate the intracellular space while increasing vascular volume and oxygen carrying capacity of the blood.

Discussion

Oncotic pressure results when two fluid compartments are separated by a membrane that is impermeable to one or more solutes in either or both compartment. The potential is for solvent to move to equalize the concentration of the impermeable solutes. This potential is equivalent to hydrostatic pressure, and thus the Starling Hypothesis proposes that mean systolic pressure in the capillary (18 to 28 mmHg) is balanced by a higher concentration of protein in the plasma than in the interstitium. This principle will apply equally across other membranes, for example the red cell membrane, however, in the complexity of the physiologic system, osmotic pressure, Gibbs-Donnan electrical forces, and the active cation pumps, as well as chemical reactions and possible cell volume homeostatic control systems, are also present, so that permeable solutes and the solvent must transport to satisfy several constraints simultaneously.

Frequently this complex balance of forces is upset, either pathologically or iatrogenically. Therapies consisting of NS-D5 or NS-D10, being respectively two and three times normal osmolarity, temporarily expand the vascular volume until the glucose, with a half-time of 15-20 minutes, can diffuse into the interstitium and be taken up by the cells. Hypertonic saline solutions, now under study, act similarly but with a shorter half-time, 5-10 minutes. Water diffuses along its osmotic concentration gradient very quickly, with half-times on the order of less than one minute (as can be noted in the time required to lyse red cells in distilled water) so that with respect to most solutes, water is essentially in osmotic equilibrium, that is, as Na+ or glucose move across membranes, sufficient water goes along to maintain osmotic equilibrium. Glucose tolerance tests will raise blood sugar to 200 mg/dl, about 10 meq/l of temporary onconicity (and diabetics may present with sugar levels of 500 mg/dl). While this test sugar is excluded from the cells, it will exercise oncotic pressures of 10 times that of normal plasma protein. Now these situations may not be innocuous (particularly if chronic), although under normal circumstances we believe them to be transient. By

comparison, the DBBF perfusions are not out of the range of usual procedures. Hyperoncotic, cross-linked hemoglobin tetramer solutions of 14 mg/dl are only slightly over 2 mosm, on the order of twice that of normal plasma protein and less than half the concentration of Hb in erythrocytes. But, again, several forces are simultaneously at play, other than onconicity, so the result of a given procedure under particular circumstances is not immediately obvious

Although absolute oncotic pressures can be quite high-- about 2C +0.2C2 0.01C3, where C is protein in gm/dl (Guyton, 1980)-- the cells are partially buffered or "protected" from oncotic stress by the fact that, through the agency of the cation pump, Na is excluded from the cell, i.e., acts like an oncotic particle. The sum of all of the "oncotic" solutes both inside and outside of the cell is thus very high and the relative change in onconicity by the change of a few mosm outside will have a proportionately smaller effect on the cell. The Gibbs-Donnan forces (electrical neutrality) also play a role (e.g., forcing K into, and mobile anions out of the cell), and it is believed that the cell also responds by allocating a variable amount of energy to the active pump (with the tendency to maintain cell volume). Hydrostatic pressure has no direct effect on cell volume because it is transmitted through the cell wall without loss, so that if the cellular oncotic, osmotic, charge, and cation pump forces are not in balance, the cell volume will change.

The erythrocytes are more vulnerable than, say, muscle or neural cells because of their simpler structure and because they contain far less fixed negative ions, and therefore more mobile ions such as Cl- and HCO3- that can transport in response to Gibbs-Donnan forces . This effect can be noted in the apparently anomalous behavior of the red cells in some experiments of this paper, especially in the hypertonic experiments of Table 4 where, without volume regulation (which is not present in this simulation), the red cell volume increases by a factor of three. No doubt this is an exaggeration of the true state, but the concave discoid red cell volume can change by a factor of two without changing the measured hematocrit appreciably and a factor of five before it lyses.

However, with the exception of the anomalous red cell, the volume increment of the intracellular space in the situations posed herein is relatively very small, never more than 10 percent; the clinical problem, if any, with the high onconicity fluid therapies must be in the vascular or interstitial expansion, which are evidently only the order of fifty percent, not particularly high compared to a variety of clinical problems. Clinically, medium dehydration or medium over-hydration occurs at 10 percent of total body weight, which in the case of these animals would be 2.2 liters, or seven liters in the 70 Kg man, and the patient is ill but responsive. As in Table 4 where

the loss is 1.5 liters of 35 meq/l saline, such a loss involves all compartments of the body and is usually completely recoverable.

A theoretical simulation can aid study of this complex system by demonstrating the qualitative as well as quantitative aspects of the fluid stress. For example, hemorrhage case, Table 5, the total loss is about 825 ml of blood, and, though it is not shown, 0.825 liters of whole blood (given immediately) would simply restore the system to normal values. Ringer's alone is given to restore vascular volume, but, since it diffuses into the interstitium (half-time about 15 min, Wolfe, 1986), additional volume must be given to maintain vascular volume or systolic pressure. Concentrated serum albumin is fluid of choice to reduce the volume of interstitium, a functional behavior that is well known, but as a reperfusion fluid for hemorrhage the interstitium may already be relatively down and it can draw very little volume from the cells because of the protein negative charge.

Concentrated saline, now under study, may be a valuable addition to the fluid management tools but for the possible dangers of elevated plasma sodium and sharply decreased cell volume. Also, the initial effect of concentrated saline is much more drastic; water diffuses much more rapidly than Na+ or Cl-, so before the salt diffuses into the interstitium the high saline concentration in plasma will increase the plasma volume to 2.5 liters, where the normal volume is 1.1 liters. The dextran has a pure oncotic effect compared to serum albumin, that is, it is without the additional charge effect of albumin or DBBF, therefore its global effect is quite different, for example, the expansion of the red cells with dextran is due solely to osmotic dilution; the tonicity of the plasma decreases under dextran.

The clinical research problem addressed here is whether the sum of these physical forces brought to play by the administration of DBBF solutions, at concentrations slightly over twice that of normal plasma proteins, is innocuous. In the cases examined, plasma volume expansion by the DBBF perfusion is generally on the high side of the distribution of volumes created by alternative therapies. On the other hand, the expansion of interstitial space is generally lesser and the effect on intracellular space, which probably should be protected, is minimal. In no case are the volumes (or electrolyte concentrations) out of the bounds of normal clinical experience.

The DBBF exchange transfusion protocol is not likely to have clinical application, but it does demonstrate the survivability of the animal on the cross-linked hemoglobin solution. The objective of providing adequate oxygen carrying capacity is also fully realized; none of the DBBF trials resulted in capacity less than normal. This fact, plus its low viscosity and lack of immune response, opens the potential for use of

the DBBF as a blood substitute in procedures other than hemorrhage, for example in liver transplants or emergency transfusions.

While the physical forces described here can be simulated on a computer and the fluid and electrolyte changes predicted, it is not possible with assurance to predict the many potential physiological side-effects consequent from osmotic or oncotic stress, such as inotropic stress on the heart or osmotic stress on the brain. However, if the compartmental membranes are patent, not modified by toxic, inflammatory or pyrogenic reactions, and if the protein can be catabolized innocuously, it is likely that the physiology is not harmed by temporary, expansions of vascular or interstitial spaces on the order of those seen in the current DBBF protocols.

Table 1. Observed and calculated blood hemoglobi n during reperfusion, gm/dl

Aliquot	Comp Pl Hb	Comp RC Hb	Obs RC	Obs Pl Hb
0	0	8.746	8.839	0
1	.623	8.397	8.305	0.83
2	1.208	8.057	7.677	1.84
3	1.758	7.727	7.253	2
4	2.274	7.408	6.751	2.5
5	2.756	7.099	6.327	2.9
6	3.203	6.799	5.872	3.2
7	3.619	6.51	5.793	3.6
8	4.002	6.23	5.574	4.1
9	4.353	5.96	5.212	4.3
10	4.672	5.7	4.82	4.6
11	5.011	5.501	4.726	5
12	5.264	5.251	4.302	5.3
13	5.491	5.012	4.223	5.1
14	5.689	4.782	3.988	5.7
15	5.862	4.561	3.894	5.6
16	6.008	4.349	3.485	5.7
17	6.131	4.146	3.485	6.1
18	6.484	3.753	3.25	6.2
20	6.334	3.574	3.093	6.3
21	6.364	3.403	2.999	6.5
23	6.359	3.083	2.795	6.6
24	6.287	3.001	2.795	6.5
24	6.325	2.934	2.763	6.6
25	6.272	2.791	2.622	6.6

Table 2. Control data; reperfusion with DBBF solution.

	Normal Control	Observed Hannon	down 70% bl	perfuse pig4p4	remove plasma	glucose 500 mg%
plvol	1.110	1.1	.875	1.877	1.294	1.893
rcvol	.411	.41	.123	.386	.366	.333
isvol	2.171	2.2	2.166	2.406	2.453	1.786
icvol	8.338	8.3	8.325	8.224	8.219	8.270
hct	27.05	27.0	12.34	17.06	22.07	14.97
blvol	1.52	1.52	1.00	2.26	1.66	2.23
plhbx	.00	.00	.00	9.00	13.05	.00
tblhb	8.75	8.7	4.00	9.23	12.57	14.09
plNa+	145.	142.	145.	142.	139.	130.

Table 3. Compare DBBF reperfusion with miscellaneous observations.

	perfuse pig4p4	0 chg on DBBF	Cl/HCO3 pH=7.43	1 liter Ringers	250 ml 25% SA	250 ml 1M NaCl
plvol	1.877	1.845	1.770	1.130	1.506	1.488
rcvol	.386	.292	.106	.274	.272	.317
isvol	2.406	2.504	2.550	2.821	2.620	3.543
icvol	8.224	8.250	8.380	8.265	8.374	7.478
hct	17.06	13.68	5.63	19.53	15.32	17.56
blvol	2.26	2.14	1.88	1.40	1.78	1.80
plhbx	9.00	9.15	9.54	.00	.00	.00
tblhb	9.23	9.77	11.13	2.84	2.24	2.21
plNa+	142.	140.	146.	146.	148.	165.

Table 4. Comparison of DBBF reperfusion with treatment of hypertonicity.

	Control	loss1.5 l 1/4NS	add 1.5 Ringers	250 ml 25% SA	250 ml 1M NaCl	1 liter DBBF
plvol	1.108	.810	1.222	1.576	1.592	1.699
rcvol	.409	.887	1.081	1.099	1.025	1.042
isvol	2.168	1.564	2.407	2.244	3.024	1.931
icvol	8.331	7.360	7.395	7.494	6.799	7.387
hct	26.95	52.26	46.93	41.08	39.17	38.01
blvol	1.52	1.70	2.30	2.68	2.62	2.74
plhbx	.00	.00	.00	.00	.00	10.30
tblhb	8.77	7.84	5.78	4.97	5.08	11.24
plNa+	145.	172.	167.	168.	186.	162.

Table 5. Comparison of DBBF reperfusion with treatment of hemorrhage.

	Control	loss 37 ml/Kg	1 liter Ringers	250 ml 25% SA	250 ml 1M NaCl	1 liter DBBF
plvol	1.108	.513	.686	1.136	.961	1.694
rcvol	.409	.189	.302	.325	.359	.477
isvol	2.168	2.162	2.925	2.659	3.744	2.456
icvol	8.331	8.315	8.283	8.388	7.470	8.309
hct	26.95	26.95	30.54	22.25	27.21	15.41
blvol	1.52	.70	.99	1.46	1.32	2.00
plhbx	.00	.00	.00	.00	.00	10.33
tblhb	8.77	8.79	6.25	4.23	4.68	11.62
plNa+	145.	145.	146.	148.	165.	140.

Table 6. Compare DBBF reperfusion with toploading the pig.

	Control	250 ml 25% SA	250 ml 1M NaCl	1M NaCl w/o dex	1 mosm dextran	1 liter DBBF
plvol	1.110	1.454	1.536	1.488	1.270	1.932
rcvol	.411	.384	.390	.390	.403	.477
isvol	2.171	2.018	2.910	2.957	2.097	2.456
icvol	8.338	8.468	7.537	7.537	8.495	8.309
hct	27.05	20.88	20.24	20.76	24.08	19.80
blvol	1.52	1.84	1.93	1.88	1.67	2.41
plhbx	.00	.00	.00	.00	.00	7.25
tblhb	8.77	7.24	6.92	7.08	7.90	11.34
plNa+	145.	147.	165.	166.	141.	145.

Fig. 1. Observed and Computed Reperfusion Hemoglobin.

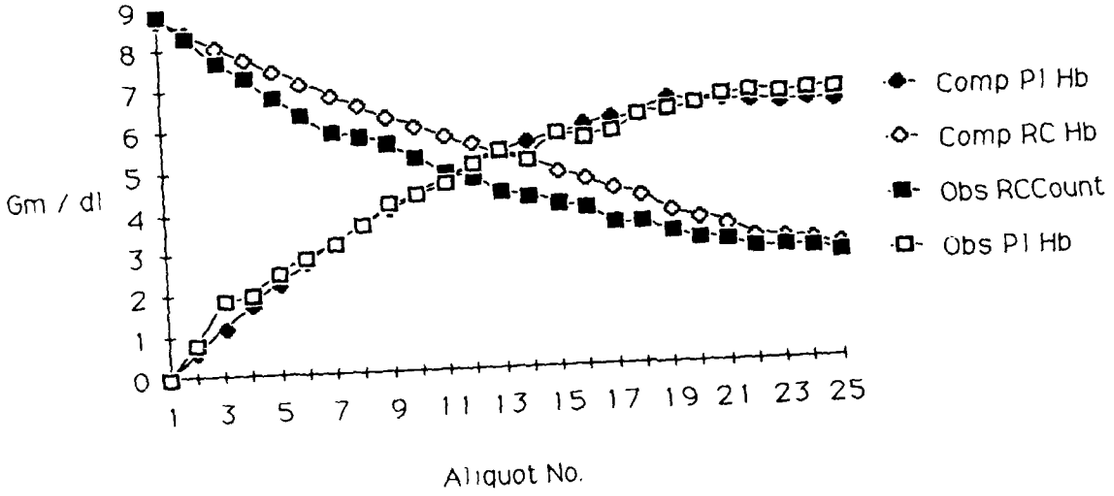


Fig. 2. Control data; reperfusion with DBBF solution.

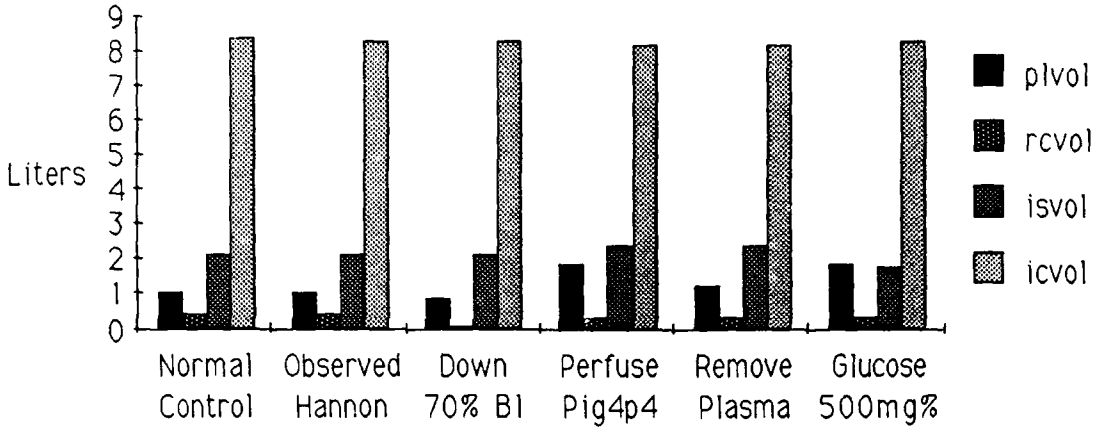


Fig. 2a. Control data; reperfusion with DBBF solution.

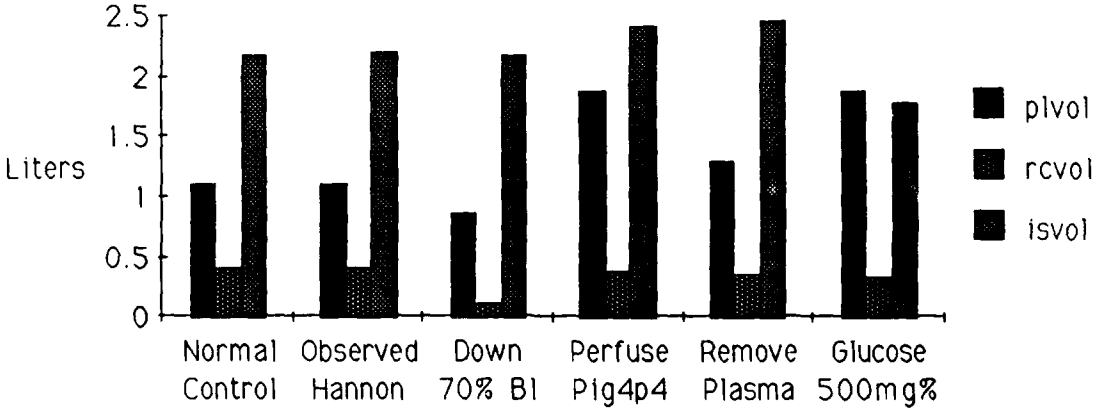


Fig. 3. Compare DBBF reperfusion with misc. observations.

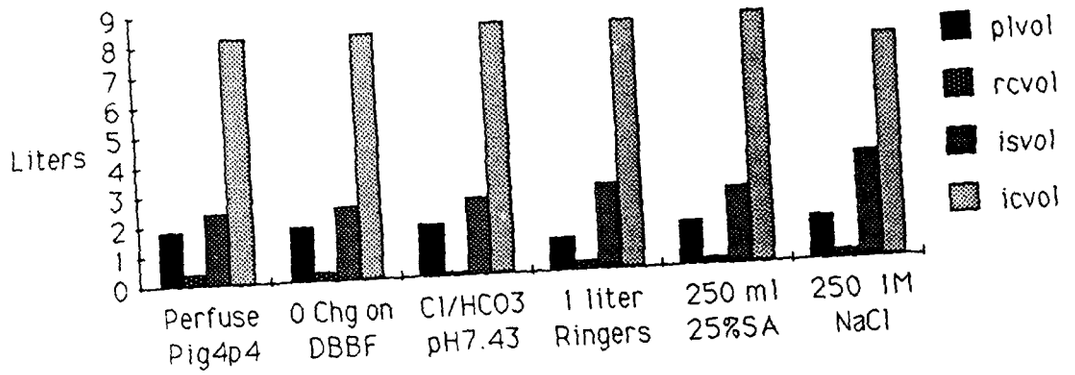


Fig. 3a. Compare DBBF reperfusion with misc. observations.

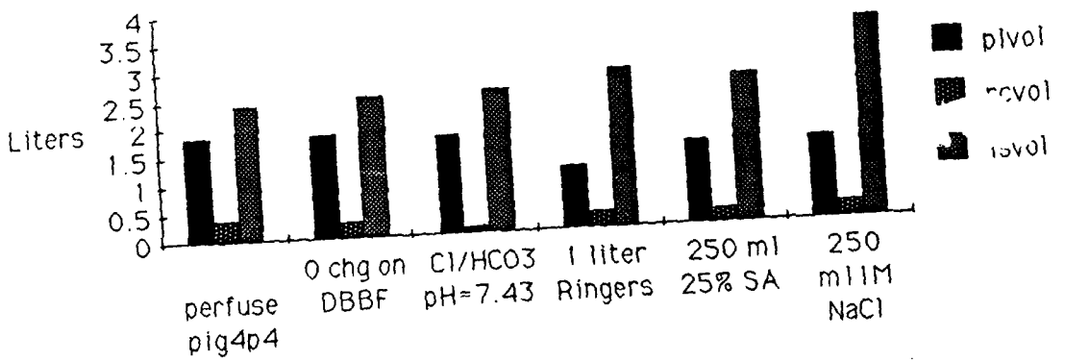


Fig. 4. Compare DBBF reperfusion with misc. observations.

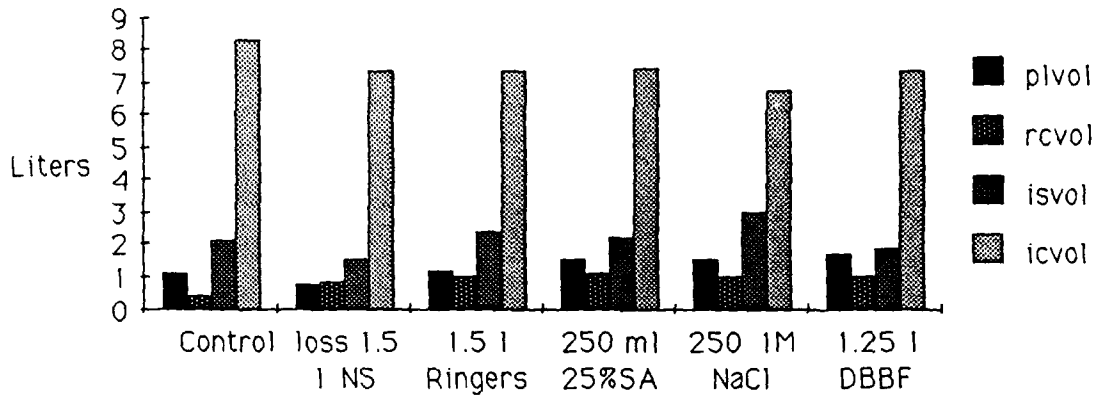


Fig. 4a. Compare DBBF reperfusion with misc. observations.

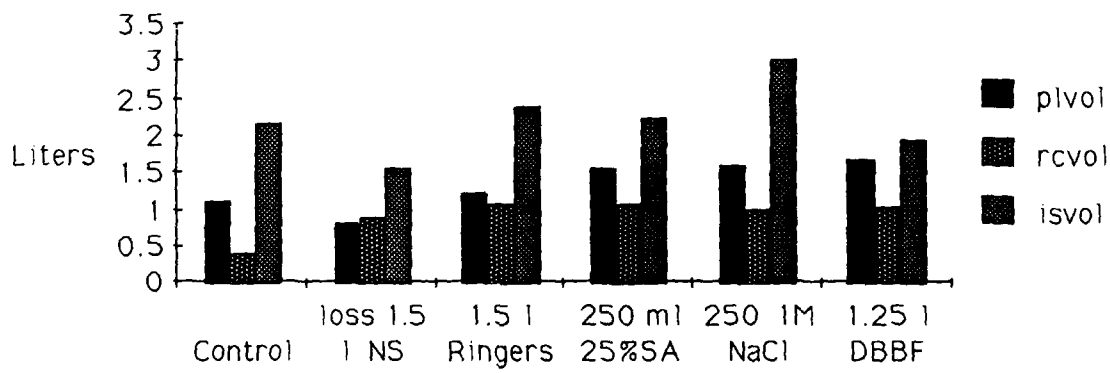


Fig. 5. Comparison of DBBF reperfusion with treatment of hemorrhage.

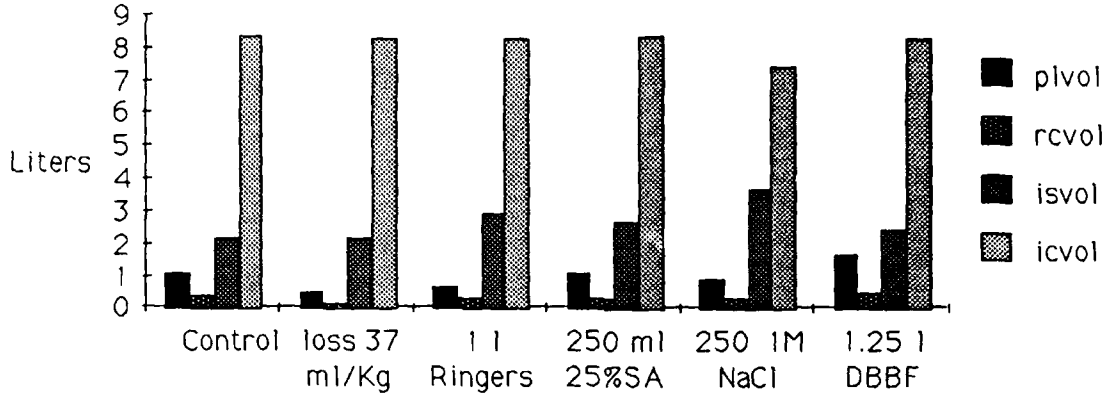


Fig. 5a. Comparison of DBBF reperfusion with treatment of hemorrhage.

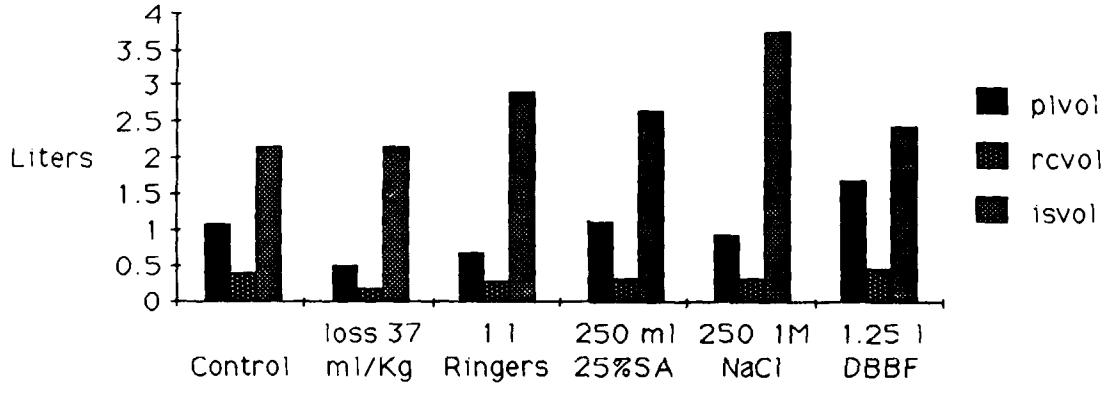


Fig. 6. Comparison of DBBF reperfusion with toploading the pig.

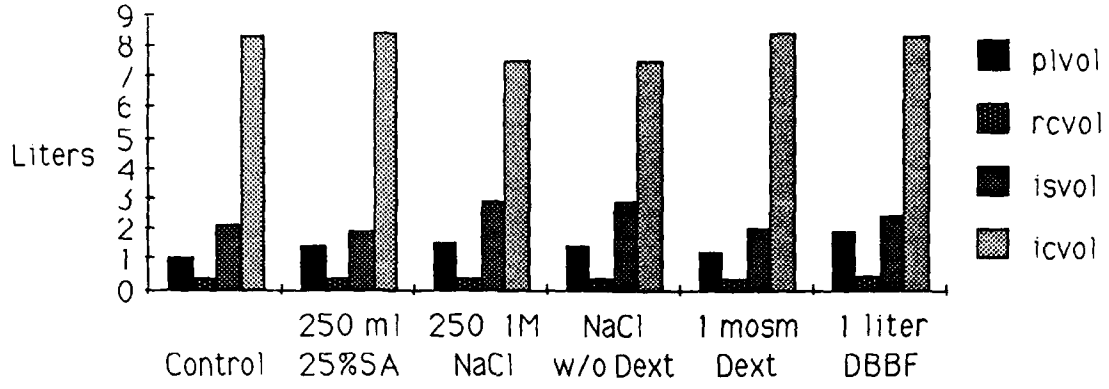
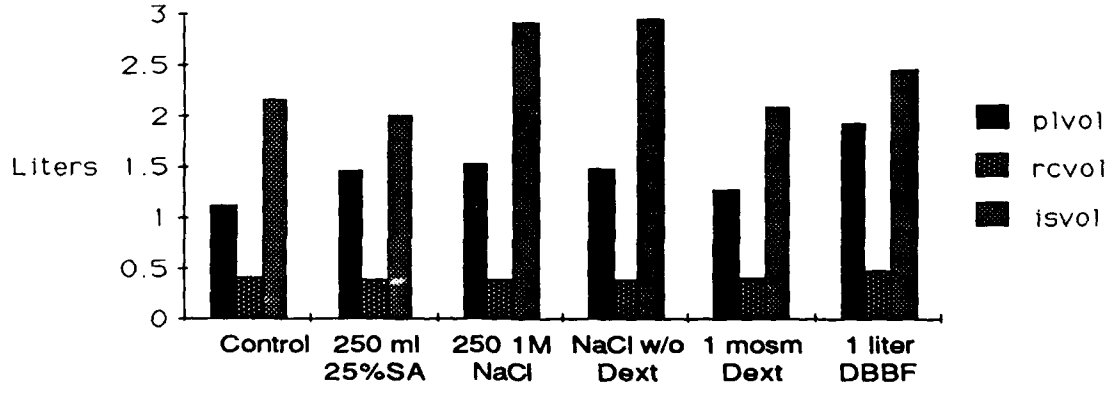


Fig. 6a. Comparison of DBBF reperfusion with toploading the pig.



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